Available online at www.sciencedirect.com
ScienceDirect
Journal of Forensic and Legal Medicine 15 (2008) 205–209
JOURNAL OF FORENSIC AND LEGAL MEDICINE
www.elsevier.com/jflm

Review

Marfan syndrome and sudden death within a family – Aetiologic, molecular and diagnostic issues at autopsy

Rena Hirani PhD [a], Barbara Koszyca MBBS, PhD [a,b], Roger W. Byard MBBS, MD [a,b,*]

[a] *Discipline of Pathology, Level 3 Medical School North Building, University of Adelaide, Frome Road, Adelaide 5005, South Australia, Australia*
[b] *Forensic Science SA, Adelaide 5005, South Australia, Australia*

Received 29 April 2007; accepted 15 July 2007
Available online 1 November 2007

Abstract

Although Marfan syndrome has a range of characteristic morphological features involving the ocular, cardiovascular and musculoskeletal systems, the phenotype is variable. In addition, mutations have been identified in the gene encoding for fibrillin-1 and also in the transforming growth factor-β receptor 2 (TGF-βR2) gene. Two cases are presented of sudden and unexpected deaths in cousins who manifested morphologic features of Marfan syndrome at autopsy. Case 1: A 36-year-old male who collapsed and was found at autopsy to have arachnodactyly, a high arched palate and lethal aortic dissection with haemopericardium. Case 2: A 34-year-old male who collapsed and was found at autopsy to have arachnodactyly, a high arched palate, pes cavus and a dysplastic mitral valve. Current aetiological theories and molecular findings are discussed. While family follow-up and counselling are advised when cases come to autopsy, given the variability in phenotype and genotype, and the difficulties that exist in attempting to determine clinical prognosis from either of these, such deaths may raise more concerns for surviving family members than providing answers.

Keywords: Marfan syndrome; Sudden death; Family counselling; Fibrillin; Transforming growth factor-β

1. Introduction

Marfan syndrome (OMIM #154700) is an inherited, autosomal dominant disorder that affects the skeletal, ocular and cardiovascular systems. The disease displays high penetrance and wide clinical variability both within and between families, therefore a patient may have mild to severe symptoms that may or may not correlate with other affected family members. The skeletal abnormalities are the most readily recognisable phenotype of the disease with long bone overgrowth resulting in an unusually tall, thin individual with disproportionately long extremities and weakened musculature. Arachnodactyly, or long fingers and toes, and overgrowth of the ribs are hallmarks of Marfan syndrome patients.[1,2]

Individuals with the syndrome may be identified clinically and at autopsy by their characteristic morphology, but support for the diagnosis requires genetic testing. Two unexpected and sudden deaths of young males within the same family over a short period of time are reported to demonstrate typical features of these cases at autopsy and to illustrate problems that may arise if molecular studies and family counselling are planned.

2. Case reports

2.1. Case 1

A 36-year-old male with no significant medical history presented to his local doctor complaining of chest pain and bitemporal headaches that had been present all day. He had not experienced similar pains before and there were

* Corresponding author. Address: Discipline of Pathology, Level 3 Medical School North Building, University of Adelaide, Frome Road, Adelaide 5005, South Australia, Australia. Tel.: +61 8 8303 5441; fax: +61 8 8303 4408.
E-mail address: byard.roger@saugov.sa.gov.au (R.W. Byard).

1752-928X/$ - see front matter © 2007 Elsevier Ltd and FFLM. All rights reserved.
doi:10.1016/j.jflm.2007.07.010

no associated features. Whilst being examined by his doctor he complained that the pain was worse and then collapsed. He could not be resuscitated. An electrocardiograph taken when the deceased had arrived at the doctor's surgery was normal.

At post-mortem the body was that of a well-nourished adult white male weighing 86 kg and measuring 185 cm (body mass index, BMI = 25.1). Arachnodactyly and a high arched palate were noted, and on opening the chest there was a 900 ml haemopericardium (Fig. 1a) associated with dissection of the aortic root (Fig. 1b). The aortic root was dilated, with a diameter of 70 mm, and an 80 mm tear anteriorly with a dissection extending proximally into the pericardial sac. The heart was enlarged weighing 595 g ($N = 275$–478 gm)[3] and there was a probe patent foramen ovale. The coronary arteries were unremarkable except for an incidental 40% atherosclerotic narrowing of the left anterior descending coronary artery. The mitral valve was normal. There were no other significant findings. Histologic examination of the wall of the aorta confirmed the presence of dissection associated with degeneration of elastin fibres, fragmentation of collagen and accumulation of acid mucopolysaccharides typical of cystic medial necrosis. Death was attributed to cardiac tamponade secondary to aortic dissection in an individual with morphologic features of Marfan syndrome.

2.2. Case 2

Eight days later, a 34-year-old male cousin of the deceased in Case 1 collapsed. Attempts at resuscitation were unsuccessful. The deceased had been previously-diagnosed with mitral valve prolapse with moderate to severe mitral regurgitation and mild dilated cardiomegaly and it had been expected that valve replacement would eventually be required.

At post-mortem the body was that of a well-nourished adult white male weighing 95 kg and measuring 186 cm (BMI = 27.5). Arachnodactyly, pes cavus and a high arched palate were noted. The heart was enlarged weighing 574 g ($N = 288$–503 gm),[3] with a left ventricular wall thickness of 20 mm and a dilated left atrium. The mitral valve was markedly dysplastic with hooding and thickening of both leaflets (Fig. 2) with less severe changes in the tricuspid valve. There was no significant coronary artery disease; no aortic valve abnormality and no aortic dilatation. There were no other significant findings. Histologic examination of the mitral valve showed myxoid change with thickening of the spongiosa and increased deposition of acid mucopolysaccharides. Death was attributed to mitral valve prolapse syndrome in an individual with morphologic features of Marfan syndrome.

3. Discussion

Marfan syndrome is one of the most frequently encountered inherited disorders of connective tissues, being found in 2–3 individuals per 10,000 of the population. There is no ethnic predisposition. The first description in the medical literature was in 1896 when Marfan reported details of a tall 5-year-old girl who had long fingers and toes. The association with aortic abnormalities was first reported in

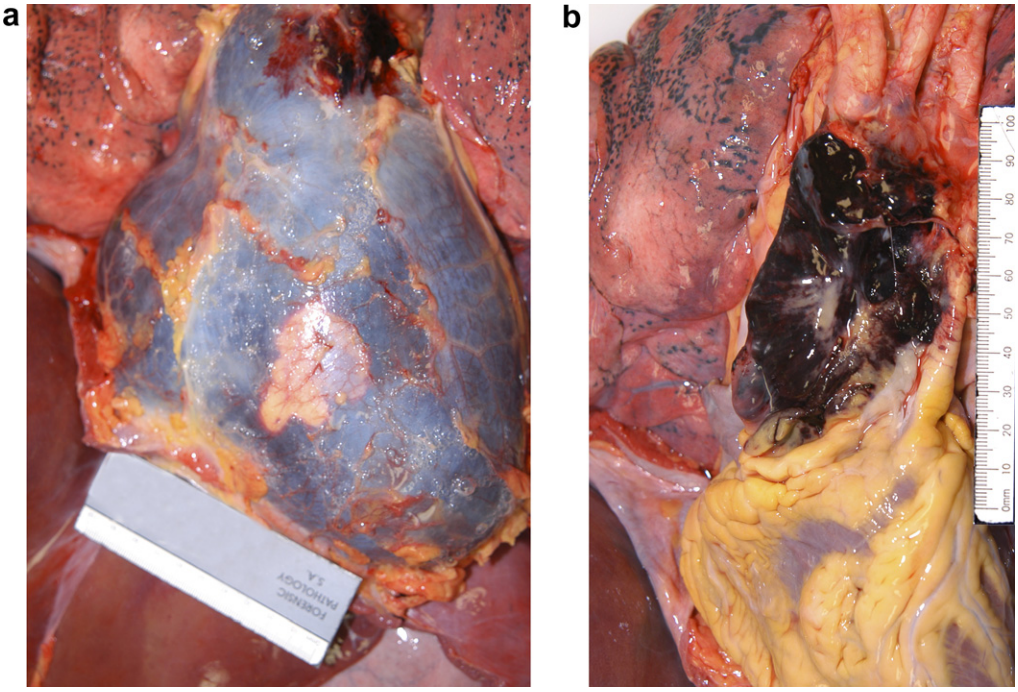

Fig. 1. Filling of the pericardial sac with 900 ml of fluid and clotted blood in a 36-year-old male with a Marfanoid habitus (Case 1) (a) was associated with dissection of the proximal ascending aorta. Interstitial and periadventitial haemorrhage surrounded the area of dissection (b).

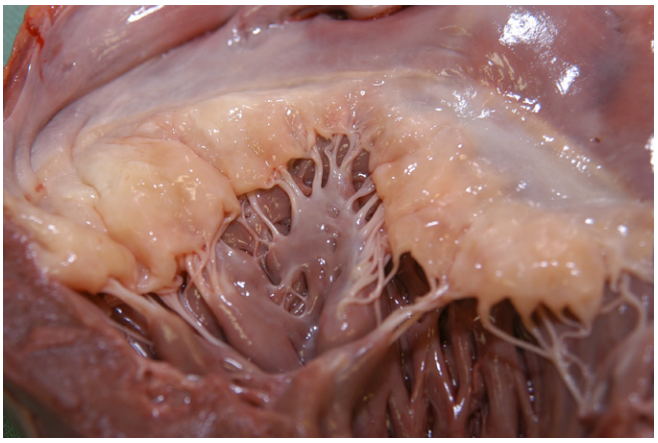

Fig. 2. Marked dysplasia of the mitral valve with thickening and hooding of both leaflets in a 34-year-old male with a Marfanoid habitus (Case 2).

1943.[1] Marfan syndrome is a multisystem disorder with considerable phenotypic variability among individuals, even within the same family. Affected individuals are typically tall and thin with an arm span to height ratio >1.05. At autopsy manifestations of Marfan syndrome may be arachnodactyly, dolichostenomyelia, kyphoscoliosis, a high arched palate, cutaneous striae and pectus excavatum or carinatum. There may also be dolichocephaly, flattened zygomas, and a retrognathic or hypoplastic mandible. On internal examination there may be a dysplastic mitral valve and dilatation/aneurysm formation of the ascending aorta. The Ghent diagnostic criteria provide guidelines for establishing the clinical diagnosis.[4]

Individuals with Marfan syndrome often present to forensic facilities because of the risk of early and sudden death, most often due to aortic dissection with haemothorax and haemopericardium (as in Case 1), with cardiac tamponade and/or coronary artery compromise. Other cardiovascular causes of premature demise include aneurysm rupture, mitral valve prolapse (as in Case 2), cardiac failure and arrhythmias and endocarditis associated with valve replacement. Fatal or near-fatal events due to these conditions may be precipitated by exercise, trauma, stimulant usage and pregnancy, and may occur at very early ages including infancy.[5] Deaths may also result from atlanto-occipital instability or from post-operative complications following cardiovascular surgery. Lethal mechanisms associated with Marfan syndrome are discussed in greater detail elsewhere.[6]

In the early 1990s, the Marfan syndrome disease gene was mapped to the same chromosomal position as the *FBN1* gene (15q21.1).[7,8] Genetic linkage between *FBN1* and the Marfan phenotype was established, and over 500 mutations within *FBN1* have been subsequently identified.[9] Extensive linkage and mutation analyses have not yielded many common mutations for the classic Marfan syndrome phenotype, with most patients and/or families having unique mutations. In addition, 25–30% of cases are sporadic.

Fibrillins are a family of large (350 kDa), rod-shaped proteins that form the major structural component of elastic fibre microfibrils within many elastic/connective tissues including the aorta, the ocular ciliary zonules, tendons and skin. The biochemical pathway of fibrillin-1 assembly into microfibrils is poorly understood, and as a consequence the mechanism by which mutations in *FBN1* result in disease is unclear. It has been suggested that the major pathogenic mechanism could be a dominant negative effect of a mutant fibrillin-1 protein on microfibril assembly. Current research suggests that a critical threshold of native fibrillin-1 protein is required to form functioning microfibrils within elastic tissues. If *FBN1* mutations are introduced, this could result in the production and integration of abnormal fibrillin proteins into newly formed microfibrils thus compromising their function within the extracellular matrix. The requirement for a critical level of native fibrillin-1 has been further demonstrated using mice that were engineered to express reduced levels of native fibrillin-1 resulting in Marfan-like phenotypes.[10–12]

Fibrillins contain 47 tandemly repeated calcium-binding epidermal growth factor-like (cbEGF) domains that are interspersed by characteristic 8-cysteine-containing (8-Cys) motifs.[13] A large number of mutations causing Marfan syndrome affect the conserved cysteine residues within the cbEGF domains and the 8-Cys motifs. Mutations are believed to cause misfolding of these domains resulting in enhanced protein degradation and/or cleavage. Furthermore, substitution of other key structural residues, such as the highly conserved glycines, may also cause a misfold in the cbEGF and 8-Cys domains resulting in overall protein instability. Certain cysteine and glycine mutations have been studied in great detail as they have been found to be among the most common mutations in Marfan syndrome patients. Such mutations may include; C1117Y or C1129Y, which result in the accumulation of fibrillin-1 within fibroblast cells influencing the trafficking of the protein to extracellular sites for microfibril assembly, or G1127S, which causes a moderate change in the folding of a cbEGF domain and effects the integrity of the consequent microfibrils produced.[14] However, apart from a cluster of mutations in a region corresponding to exons 24–32 of *FBN1*, encoding an 8-Cys motif and cbEGF domains 11–18, a definitive genotype–phenotype correlation has not been established between Marfan syndrome and the fibrillin-1 gene. Mutations in these regions are associated with the severe neonatal form of Marfan syndrome but they may also result in milder phenotypes including classic Marfan syndrome.[15–17]

In addition to the structural role of fibrillins within microfibrils, fibrillin-containing microfibrils also appear to have a critical role in the regulation and storage of cytokines. Cytokines are molecules that affect tissue development and homeostasis with a highly regulated activity within tissues. The active dimer of one such cytokine, transforming growth factor-β (TGF-β), is complexed to its latency associated protein (LAP) and one of

three latent TGF-β binding proteins (LTBPs). Removal of both the LAP and LTBP is required for the active TGF-β dimer to be released. Upon secretion, the latent TGF-β complex is targeted to specific sites within the extracellular matrix and recent data have indicated that one such target region is fibrillin-containing microfibrils. Specifically it has been shown that fibrillin-1 interacts with TGF-β-bound LTBPs-1 and -4.[18] Loss of fibrillin-1-containing microfibrils in Marfan syndrome leads to multiple phenotypic features that may result from aberrant TGF-β activation. It has also been shown that mice deficient in fibrillin-1 exhibit marked dysregulation of TGF-β activation and signalling, resulting in apoptosis within the developing lung.[19] It is thus feasible that mutated fibrillins may result in reduced interaction with LTBP, thus disrupting TGF-β activation.

Further evidence to support a role for TGF-β in Marfan syndrome has recently been demonstrated within a subset of patients, who have negative *FBN1* gene screening, but who have been shown to have defects in the TGF-β receptor 2 (*TGFBR2*) gene. *TGFBR1* and *TGFBR2* mutations have also been identified in Loeys–Dietz aortic aneurysm syndrome.[20–23] *TGFBR2* belongs to the serine–threonine kinase family of cell surface receptors, which regulate several cellular processes, including proliferation, cell cycle arrest, apoptosis, differentiation and formation of the extracellular matrix.[20,24–26] The version of Marfan syndrome caused by *TGFBR2* defects is currently known as MFS2 (OMIM #154705). These patients display autosomal dominant Marfan syndrome with major cardiovascular and skeletal features, including thoracic aortic aneurysms and dissection, but without ectopia lentis. The genotyped cases studied to date are too low in number for confirmation that ectopia lentis is absent in MFS2 patients, however, this study does highlight the potential importance TGF-β in Marfan syndrome cases.[20] Mutation in *TGFBR1* has also been shown in a group of patients within the Marfan syndrome-craniosynostosis spectrum.[27]

The role of genetic testing in the diagnosis of Marfan syndrome is, therefore, currently limited as there are over 500 known fibrillin-1 gene mutations, 90% of which are unique to an individual or family. Even where affected individuals have been shown to have common mutations, there is a large amount of phenotypic variation making genotype–phenotype correlations difficult to establish. Furthermore, since *FBN1* has 65 exons, routine sequence analysis would be costly and haplotype segregation analysis would also be limiting, as 25% of patients have *de novo* mutations. There are also a number of Marfan syndrome-related disorders, which arise from *FBN* mutations. These include Shprintzen–Goldberg syndrome, familial ectopia lentis, isolated Marfanoid habitus, and Weill–Marchesani syndrome. In most cases, it is also difficult to determine the severity of each case based on the nature or location of a mutation within *FBN1*, and there are always mutations that may result in disease but that are not identified by conventional screening, especially with new gene involvements being discovered, as in the case of *TGFBR2*.

In summary, cases of previously undiagnosed or possible Marfan syndrome that are identified at autopsy may present considerable difficulties. While death can be readily attributed to aortic dissection or mitral valve syndrome, in an individual with a Marfanoid habitus, as in the reported cases, confirming the diagnosis may be difficult due to the problems with molecular testing that have been outlined. Medical evaluation of the family of the deceased is advisable and this can be undertaken through a local general practitioner, a cardiologist or through a department of medical genetics. Physical examination of family members may find Marfanoid features, and imaging and cardiological studies may identify aortic root dilatation and mitral valve disease. Phenotype within families, however, varies and this may have an effect on the significance of the physical manifestations between different individuals. Unfortunately, giving an individual family member an idea of his/her likely clinical outlook in terms of anticipated morbidity and mortality, based on the molecular identification of a particular defect in fibrillin-1 or *TGFBR* may not be possible. For this reason, while the identification of Marfanoid features and an associated cause of death at autopsy may help to resolve specific questions for a particular case, it may evoke considerable uncertainty and concerns for related family members.

Acknowledgement

We thank the South Australian State Coroner, Mr Mark Johns, for permission to report selected details of these cases.

References

1. Pyeritz RE. The Marfan syndrome. *Annu Rev Med* 2000;**51**:481–510.
2. Byard RW. Miscellaneous conditions *Sudden death in infancy childhood and adolescence*. 2nd ed. Cambridge: Cambridge University Press; 2004. p. 469–88 [chapter 12].
3. Kitzman DW, Scholz DG, Hagen PT, Ilstrup DM, Edwards WD. Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity): a quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. *Mayo Clin Proc* 1988;**63**:137–46.
4. Robinson PN, Booms P. The molecular pathogenesis of the Marfan syndrome. *Cell Mol Life Sci* 2001;**58**:1698–707.
5. Byard RW, Jimenez CL, Carpenter BF, Cutz E, Smith CR. Four unusual cases of sudden and unexpected cardiovascular death in infancy and childhood. *Med Sci Law* 1991;**31**:157–61.
6. Byard RW. Sudden death in Marfan syndrome. In: Tsokos M, editor. *Forensic pathology reviews*, vol. 4. Totowa: Humana Press; 2006. p. 93–106.
7. Pereira L, Levran O, Ramirez F, Lynch JR, Sykes B, Pyeritz RE, et al. A molecular approach to the stratification of cardiovascular risk in families with Marfan's syndrome. *N Engl J Med* 1994;**331**:148–53.
8. Boileau C, Jondreau G, Mizuguchi T, Matsumoto N. Molecular genetics of Marfan syndrome. *Curr Opin Cardiol* 2005;**20**:194–200.
9. Judge DP, Dietz HC. Marfan's syndrome. *Lancet* 2005;**366**:1965–76.

10. Pereira L, Andrikopoulos K, Tian J, Lee SY, Keene DR, Ono RN, et al. Targeting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. *Nat Genet* 1997;**17**:218–22.
11. Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, et al. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci USA* 1999;**96**:3819–23.
12. Ramirez F, Gayraud B, Pereira L. Marfan syndrome: new clues to genotype–phenotype correlations. *Ann Med* 1999;**31**:202–7.
13. Kielty CM, Sherratt MJ, Marson A, Baldock C. Fibrillin microfibrils. *Adv Protein Chem* 2005;**70**:405–36.
14. Whiteman P, Handford PA. Defective secretion of recombinant fragments of fibrillin-1: implications of protein misfolding for the pathogenesis of Marfan syndrome and related disorders. *Hum Mol Genet* 2003;**12**:727–37.
15. Kainulainen K, Karttunen L, Puhakka L, Sakai L, Peltonen L. Mutations in the fibrillin gene responsible for dominant ectopia lentis and neonatal marfan syndrome. *Nat Genet* 1994;**6**:64–9.
16. Putnam EA, Cho M, Zinn AB, Towbin JA, Byers PH, Milewicz DM. Delineation of the Marfan phenotype associated with mutations in exons 23–32 of the FBN1 gene. *Am J Med Genet* 1996;**62**: 233–42.
17. Tiecke F, Katzke S, Booms P, Robinson PN, Neumann L, Godfrey M, et al. Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype–phenotype correlations in FBN1 exons 24–40. *Eur J Hum Genet* 2001;**9**:13–21.
18. Isogai Z, Ono RN, Ushiro S, Keene DR, Chen Y, Mazzieri R, et al. Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. *J Biol Chem* 2003;**278**:2750–7.
19. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet* 2003;**33**: 407–11.
20. Disabella E, Grasso M, Marziliano N, Ansaldi S, Lucchelli C, Porcu E, et al. Two novel and one known mutation of the TGFBR2 gene in Marfan syndrome not associated with FBN1 gene defects. *Eur J Hum Genet* 2006;**14**:34–8.
21. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, et al. Aneurysm syndromes caused by mutations in the TGF-β receptor. *N Engl J Med* 2006;**355**:788–98.
22. Mátyás G, Arnold E, Carrel T, Baumgartner D, Boileau C, Berger W, et al. Identification and in silico analyses of novel TGFBR1 and TGFBR2 mutations in Marfan syndrome-related disorders. *Hum Mutat* 2006;**27**:760–9.
23. Gelb BD. Marfan's syndrome and related disorders – more tightly connected than we thought. *N Engl J Med* 2006;**355**:841–4.
24. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, et al. Heterogeneous TGFBR2 mutations in Marfan syndrome. *Nat Genet* 2004;**36**:855–60.
25. Ng CM, Cheng A, Myers LA, Martinez-Murillo F, Jie C, Bedja D, et al. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest* 2004;**114**: 1586–92.
26. Akhurst RJ. TGF-β signalling in health and disease. *Nat Genet* 2004;**36**:790–2.
27. Ades LC, Sullivan K, Biggin A, Haan EA, Brett M, Holman KJ, et al. FBN1, TGFBR1, and the Marfan-craniosynostosis/mental retardation disorders revisited. *Am J Med Genet* 2006;**140**:1047–58.